Severely dysregulated disposal of postprandial triacylglycerols exacerbates hypertriacylglycerolemia in HIV lipodystrophy syndrome[1–4]

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ABSTRACT

Background: The pathogenesis of hypertriacylglycerolemia, a characteristic feature of HIV lipodystrophy syndrome (HLS), is incompletely understood. One mechanism is accelerated lipolysis in the fasted state, but the severity of the hypertriacylglycerolemia suggests that additional underlying abnormalities may exist in the disposal of dietary fat.

Objective: Our objective was to investigate abnormalities in dietary fat disposal in the pathogenesis of hypertriacylglycerolemia in HLS.

Design: We studied 6 nondiabetic men with HLS and 6 men without HIV matched for age and body mass index as control subjects for 8 h after consumption of an isocaloric meal containing 2 g labeled $[^{13}C_3]$tripalmitin. Chylomicron-triacylglycerol disposal was estimated from labeled $[^{13}C_1]$palmitate in the plasma chylomicron fraction, and $[^{13}C_1]$palmitate oxidation was estimated from the $^{13}CO_2$ enrichment in the breath and CO_2 production, over 8 h after the meal.

Results: HLS patients had significantly elevated concentrations of fasting plasma triacylglycerols in both chylomicron ($\bar{x}$ + SE: 100.3 ± 49.5 compared with 29.2 ± 2.2 mg/dL; $P < 0.01$) and VLDL (82.4 ± 39.0 compared with 10.8 ± 2.8 mg/dL; $P < 0.01$) fractions. Chylomicron-triacylglycerol–derived $[^{13}C_1]$palmitate disposal was markedly lower in the HLS patients (3.09 ± 0.41 compared with 6.42 ± 0.18 mmol $[^{13}C_1]$palmitate/8 h; $P < 0.001$) in the 8-h postmeal period. Further, HLS patients had lowered storage of chylomicron-triacylglycerols (0.74 ± 0.38 compared with 5.05 ± 0.16 mmol; $P < 0.0001$) and elevated plasma $[^{13}C_1]$palmitate concentrations (2.01 ± 0.27 compared with 1.18 ± 0.16 mmol; $P < 0.05$) 8 h after the meal.

Conclusions: Patients with HLS have key defects that markedly impair postprandial disposal and storage of chylomicron-triacylglycerols. These defects contribute significantly to hypertriacylglycerolemia in HLS.   *Am J Clin Nutr* 2005;81:1405–10.

KEY WORDS   Lipoprotein lipase, lipolysis, fat oxidation, cholesteryl ester

INTRODUCTION

HIV lipodystrophy syndrome (HLS) is characterized by centripetal fat redistribution, dyslipidemia, and insulin resistance, suggesting that it is an accelerated form of the metabolic syndrome. HLS is associated with increased cardiovascular risk (1–7). The hypertriacylglycerolemia, which is frequently severe, is not fully responsive to standard lipid-lowering therapies, suggesting that unique mechanisms might underlie its pathogenesis. We previously reported that a pair of tandem defects in lipid metabolism could elevate VLDL-triacylglycerol concentrations in patients with HLS in the fasted state. Specifically, patients with HLS have accelerated rates of fasting lipolysis, which, coupled with the absence of a compensatory increase in lipid oxidation, increases free fatty acid (FFA) availability for hepatic re-esterification and secretion as VLDL (8). Other investigators have also reported accelerated fasting lipolysis in these patients (9–11). However, these abnormalities in the fasted state do not fully account for the magnitude of hypertriacylyglycerolemia associated with HLS. We, therefore, hypothesized that patients with HLS might have an added defect in the disposal of dietary lipids, further contributing to the hypertriacylglycerolemia of HIV lipodystrophy. To test this hypothesis, we studied patients with HLS and matched, non-HIV control subjects in the fed state, after ingestion of a meal containing 2 g $[^{13}C_3]$tripalmitin, to evaluate the disposal kinetics of dietary triacylglycerols.

SUBJECTS AND METHODS

Subjects

The study was approved by the Baylor College of Medicine Institutional Review Board for human subject research. HLS was

TABLE 1
HIV and lipodystrophy variables in subjects with HIV lipodystrophy syndrome

Patient no.	CD4 count	Viral load	Lipodystrophy scale[1]	HAART[2]
	cells/cm²	*RNA copies/cm²*		
1	491	< 400	A3B2C1E2F2	ICNR
2	205	< 400	A2B0C1E2F1	NeZL
3	503	< 400	A3B1C1E3F3	SLR
4	605	< 400	A2B0C0E3F3	ISDR
5	255	214818	A3B3C2E3F3	IE
6	320	< 400	A3B2C3E2F2	IS

[1] Letters refer to the following body regions: A, abdominal obesity; B, buffalo hump; C, supraclavicular fat pad; E, extremity fat loss; F, facial fat loss. In each region, numbers refer to severity: 0, no change; 1, mild change; 2, moderate change; 3, severe change.

[2] Highly active antiretroviral therapy. Letters refer to the following drugs: I, indinavir; C, combivir (Z/L); D, didanosine; E, efavirenz; L, lamivudine; N, nevirapine; Ne, nelfinavir; R, ritonavir; S, stavudine; Z, zidovudine.

defined by the following criteria: *1*) change in body habitus, consisting of fat loss in the extremities and increased abdominal girth, as observed by the patient and confirmed by the primary physician; *2*) lipodystrophy score, based on morphologic abnormalities in each of 5 body regions, as assessed by a single investigator [A, abdominal obesity; B, "buffalo hump" (posterior cervical fat pad); C, supraclavicular fat pad; E, extremity fat loss; F, facial fat loss]; a 4-point intensity scale was used (0 = no change; 1 = mild change; 2 = moderate change; 3 = severe change), and a score of ≥2 in at least 2 regions was required (8); *3*) fasting plasma triacylglycerol concentration ≥ 200 mg/dL. All subjects with HLS had the "mixed" phenotype of HIV lipodystrophy (peripheral fat atrophy and central adiposity) and had had no HIV-associated opportunistic infections or illnesses for ≥5 y. Six healthy men without HIV and matched for age and body mass index were recruited as control subjects. All subjects had sedentary lifestyles (exercising <2 times/wk), and none consumed unusual diets or dietary supplements. All subjects were free of diabetes mellitus, thyroid disorders, hypercortisolemia, liver or renal impairment, and hypogonadism.

HIV RNA viral load and CD4 counts were measured in all patients with HLS. Five patients had been on stable, continuous highly active antiretroviral therapy (HAART) for ≥2 y and had undetectable HIV-1 RNA concentrations (<400 copies/mL) at the time of the study. One patient (subject 5 in **Table 1**), with an elevated HIV-1 RNA concentration, had briefly interrupted HAART 3 mo before the study and had been placed on a new regimen 2 mo before the study.

Study design and experimental protocol

The study was performed in the adult General Clinical Research Center (GCRC), Baylor College of Medicine. All lipid-lowering medications were discontinued 2 wk before the study. For 2 d preceding the study, the subjects consumed a standard stabilizing diet that provided 25 kcal and 1 g protein · kg body weight^{-1} · d^{-1}. The meals were prepared in the GCRC kitchen and provided to all subjects. The outcome variables were chylomicron-triacylglycerol concentrations,

VLDL-triacylglycerol concentrations, [$^{13}C_1$]palmitate clearance from plasma, disposal of [$^{13}C_1$]palmitate from the chylomicron pool over 8 h, total CO_2 production, [$^{13}C_1$]palmitate oxidation, and calculated rates of nonoxidative [$^{13}C_1$]palmitate disposal.

After a 10-h overnight fast, baseline blood and breath samples were collected at 0700, and the subjects ingested a 2-g bolus of 1,1,1-[$^{13}C_3$]tripalmitin thoroughly mixed into a 240-mL liquid meal (Ensure; Abbott, Abbott Park, IL), containing 250 calories (6 g fat, 40 g total carbohydrates, 9 g protein). Additional blood samples were collected every 30 min for the first 4 h and then hourly for the remaining 4 h of the experimental period. Breath samples were collected every 20 min throughout the experimental period. We used a Deltatrac machine (Sensormedics, Fullerton, CA) to perform indirect calorimetry for 30 min every 2 h from the first hour (total of 4 sessions during the 8-h study period).

Sample analyses

Plasma glucose concentrations were measured by the glucose oxidase method, and plasma insulin concentrations were measured by radioimmunoassay (Linco, St. Louis, MO). The chylomicron lipoprotein subfraction was immediately separated in duplicate from the plasma samples by serial ultracentrifugation according to the method described by Rifai et al (12). Briefly, the plasma samples were overlaid with a mock solution (11.42 g NaCl, 0.1 g NaEDTA in 1 L distilled water, δ = 1.0063) in Beckman centrifuge tubes and subjected to ultracentrifugation at 33 500 × *g* for 30 min at 4 °C in a Beckman TL-100 tabletop ultracentrifuge with a TLA-100.3 rotor (Beckman Coulter Inc, Fullerton, CA). The chylomicron layer was removed carefully with a pipette. One aliquot of the chylomicron subfraction was used to measure triacylglycerols enzymatically with the use of a commercial kit (Wako, Baltimore, MD). Another aliquot of the chylomicron subfraction was subjected to thin-layer chromatography (TLC) after lipid extraction by Folch's method (13). The triacylglycerol layer was identified by fluorescence in ultraviolet light with a 0.1% solution of 2',7'-dichlorofluorescein, separated, and hydrolyzed with the use of 15% methanolic KOH to release the fatty acids. Tracer-to-tracee ratios of palmitate derived from the chylomicron-triacylglycerol fraction were determined by negative chemical ionization gas chromatography–mass spectrometry with the use of a Hewlett-Packard 5989B gas chromatography–mass spectrometry system (Fullerton, CA). The pentafluorobenzyl derivative was prepared and analyzed by selectively monitoring ions with mass-to-charge (*m:z*) ratios of 255 and 256. Breath $^{13}CO_2$ content was determined by gas isotope ratio mass spectrometry on a Europa Tracermass Stable Isotope Analyzer (Europa Scientific, Crewe, United Kingdom), monitoring ions of *m:z* 44 and 45.

Calculations

Disposal of [$^{13}C_1$]palmitate (mmol/8 h)

$$= \text{total } [^{13}C_1]\text{palmitate dose}$$

$$- [^{13}C_1]\text{palmitate remaining in plasma}$$

chylomicron-triacylglycerol after 8 h   (*1*)

The $[^{13}C_1]$palmitate dose was calculated as

$$\text{Tripalmitin dose} \times 3$$
$$\times \text{ isotopic enrichment of } [^{13}C_3]\text{tripalmitin}$$
$$\times \text{ absorption (assumed as 99\%)} \quad (2)$$

The $[^{13}C_1]$palmitate remaining in plasma chylomicron-triacylcerol after 8 h was calculated as

$$\text{CM palmitate concentration}$$
$$\times \text{ isotopic enrichment of } [^{13}C_1]\text{palmitate}$$
$$\times \text{ plasma volume} \quad (3)$$

where plasma volume was calculated from ideal body weights.

$[^{13}C_1]$Palmitate oxidation rate (mmol/8 h)

$$= \text{AUC } ^{13}CO_2 = \text{AUC}[(\dot{V}CO_2/0.78) \times \text{IE } CO_2] \quad (4)$$

where $\dot{V}CO_2/0.78$ is the production rate of CO_2 assuming that 22% of CO_2 is retained in the body's bicarbonate pool, IE CO_2 is the isotopic enrichment of CO_2 (atom% excess), and AUC is area under the curve.

$[^{13}C_1]$Palmitate storage

$$= [^{13}C_1]\text{palmitate disposal from the chylomicron fraction}$$
$$- [^{13}C_1]\text{palmitate oxidation}$$
$$- \text{nonesterified } [^{13}C_1]\text{palmitate in the plasma at 8 h} \quad (5)$$

Statistical analysis

Group data were compared by using paired t test with the use of the GRAPH PAD STATISTICAL SOFTWARE (Version 4.01; San Diego, CA). Differences were considered significant at $P < 0.05$. Data are expressed as means ± SEs.

RESULTS

All the subjects with HLS were receiving HAART (Table 1). The RNA viral load was <400 copies/mL in all subjects, except in one who had discontinued therapy because of nongastrointestinal side effects and had been restarted on a different, stable regimen 2 mo before the study. With the exception of this participant, all subjects had continued taking a stable treatment regimen for at least 6 mo. The CD4 count was below the normal range (≥500 cells/mm^3) in 4 patients. Lipodystrophy score assessment (8) revealed that all patients had peripheral and facial fat loss and abdominal obesity, and 4 patients presented abnormalities in every region (Table 1).

Compared with the control subjects, the HLS group had higher plasma concentrations of fasting total cholesterol ($P < 0.05$), triacylglycerols ($P < 0.05$), and LDL cholesterol ($P < 0.05$) and lower plasma concentrations of HDL cholesterol ($P < 0.001$). No difference was observed in concentrations of glycosylated hemoglobin between the 2 groups; however, the HLS group had significantly elevated plasma concentrations of glucose ($P < 0.05$) and insulin ($P < 0.02$) in the fasted state, implying a higher degree of insulin resistance (**Table 2**). Plasma concentrations of thyroid-stimulating hormone, free thyroxine, testosterone, and

TABLE 2
Baseline laboratory values in subjects with HIV lipodystrophy syndrome (HLS) and in non-HIV-infected control subjects[1]

Variable	HLS subjects	Control subjects
Cholesterol (mg/dL)	297.8 ± 37.3	194.7 ± 13.2[2]
Triacylglycerols (mg/dL)	488.0 ± 114.5	113.7 ± 31.5[2]
LDL (mg/dL)	172.8 ± 10.3	130.7 ± 9.2[2]
HDL (mg/dL)	28.8 ± 2.8	47.7 ± 2.8[3]
Fasting plasma glucose (mg/dL)	5.6 ± 0.1	5.2 ± 0.1[2]
Fasting plasma insulin (μIU/L)	21.6 ± 3.1	8.0 ± 1.2[2]
HbA$_{1c}$ (%)	5.0 ± 0.1	5.2 ± 0.1
AST (U/L)	30.2 ± 3.5	33.7 ± 8.6
ALT (U/L)	29.2 ± 1.9	29.7 ± 2.8
Alkaline phosphatase	63.0 ± 6.2	81.0 ± 12.7
SUN (mg/dL)	13.2 ± 1.7	15.3 ± 1.7
Creatinine (mg/dL)	1.1 ± 0.1	1.1 ± 0.1
TSH (mU/L)	2.6 ± 0.1	2.1 ± 0.3
Free thyroxine (ng/dL)	1.1 ± 0.1	1.2 ± 0.1
Testosterone (ng/dL)	634.8 ± 85.8	573.0 ± 20.2
Hemoglobin (g/dL)	15.1 ± 0.1	14.7 ± 1.6

[1] All values are $\bar{x}$ ± SEM; $n = 6$. HbA$_{1c}$, glycosylated hemoglobin; AST, aspartate transaminase; ALT, alanine transaminase; SUN, serum urea nitrogen; TSH, thyroid-stimulating hormone.
[2,3] Significantly different from the HLS group (Student's t test): [2] $P < 0.05$, [3] $P < 0.001$.

hemoglobin and indices of renal and liver function were within normal ranges in both groups.

The HLS group had markedly elevated fasting plasma concentrations of both chylomicron-triacylglycerols (100.3 ± 49.5 compared with 29.2 ± 2.2 mg/dL; $P < 0.01$) and VLDL-triacylglycerols (82.4 ± 39.0 compared with 10.8 ± 2.8 mg/dL; $P < 0.01$) (**Table 3**). The kinetics of orally administered 1,1,1-$[^{13}C_3]$tripalmitin (**Figure 1**) revealed severely impaired disposal of plasma chylomicron-$[^{13}C_1]$palmitate in the HLS group, both in absolute terms (3.09 ± 0.41 and 6.42 ± 0.18 mmol $[^{13}C]$palmitate/8 h for the HLS and control groups, respectively, $P < 0.001$) and as a percentage of the ingested dose (45.23 ± 5.93% compared with 94.01 ± 2.57%, $P < 0.001$; Table 3). The

TABLE 3
Kinetics of disposal of dietary $[^{13}C_3]$tripalmitin in subjects with HIV lipodystrophy syndrome (HLS) and in non-HIV-infected control subjects[1]

Variables	HLS subjects	Control subjects
Before test meal		
CM:TG concentration (mg/dL)	100.3 ± 49.5	29.2 ± 2.2[2]
VLDL:TG concentration (mg/dL)	82.4 ± 39.0	10.8 ± 2.8[2]
After test meal		
Disposal of $[^{13}C_1]$palmitate (mmol/8 h)	3.09 ± 0.41	6.42 ± 0.18[3]
Disposal of $[^{13}C_1]$palmitate/total dose (%)	45.23 ± 5.93	94.01 ± 2.57[3]
$[^{13}C_1]$Palmitate oxidation (mmol/8 h)	0.34 ± 0.04	0.20 ± 0.03[2]
Plasma $[^{13}C_1]$palmitate at 8 h (mmol)	2.01 ± 0.27	1.18 ± 0.16[4]
Storage of $[^{13}C_1]$palmitate at 8 h (mmol)	0.74 ± 0.38	5.05 ± 0.16[5]
Storage of $[^{13}C_1]$palmitate/total dose (%)	10.80 ± 5.58	73.90 ± 2.30[5]

[1] All values are $\bar{x}$ ± SEM; $n = 6$. CM, chylomicron; TG, triacylglycerol.
[2–5] Significantly different from the HLS group (Student's t test): [2] $P < 0.01$, [3] $P < 0.001$, [4] $P < 0.05$, [5] $P < 0.0001$.
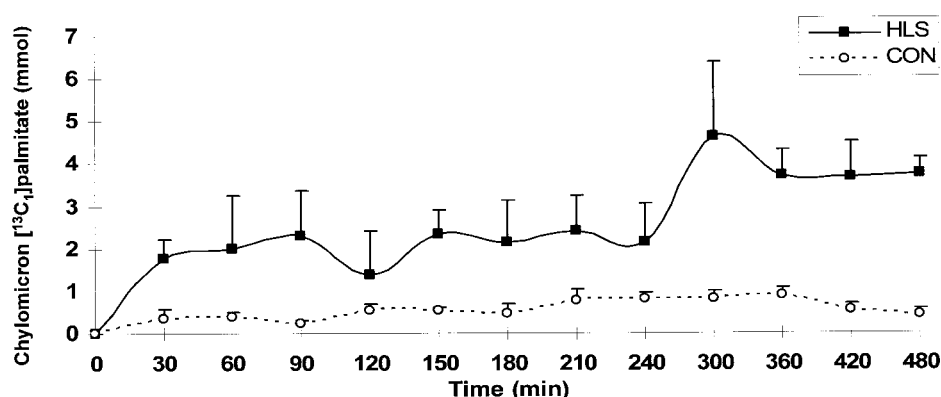
FIGURE 1. Mean ($\pm$SE) concentrations of chylomicron-derived $[^{13}C_1]$palmitate plotted over 8 h after consumption of a meal containing $[^{13}C_3]$tripalmitin. The baseline concentrations of chylomicron $[^{13}C_1]$palmitate have been normalized to zero for all subjects. Solid line, subjects with HIV lipodystrophy syndrome (HLS); broken line, control (CON) subjects without HIV.

absolute amount of nonesterified $[^{13}C_1]$palmitate in the plasma (ie, the labeled palmitate tracer derived from the ingested $[^{13}C_3]$tripalmitin), measured 8 h after consuming the meal, was significantly elevated (2.01 $\pm$ 0.27 compared with 1.18 $\pm$ 0.56 mmol; $P < 0.05$; Table 3) in the HLS subjects than in the control subjects. This greater amount of nonesterified $[^{13}C_1]$palmitate remaining in the plasma compartment of the patients with HLS was largely due to lowered storage of $[^{13}C_1]$palmitate, because net storage of the $[^{13}C_1]$palmitate tracer 8 h after consumption of $[^{13}C_3]$tripalmitin was markedly lower in the HLS group than in the control group, both as an absolute amount (0.74 $\pm$ 0.38 compared with 5.05 $\pm$ 0.16 mmol; $P < 0.0001$) and as a percentage of the total tracer dose consumed (10.80 $\pm$ 5.58 compared with 73.90 $\pm$ 2.30 mmol; $P < 0.0001$). In addition, the greater amount of nonesterified $[^{13}C_1]$palmitate in the plasma compartment of the HLS group was associated with an increased oxidation of $[^{13}C_1]$palmitate compared with the rate of the control group (0.34 $\pm$ 0.04 compared with 0.20 $\pm$ 0.03 mmol/8 h; $P < 0.01$) (Table 3).

To further assess the disposal of triacylglycerols from chylomicrons, we performed TLC to measure the proportions of triacylglycerols to cholesteryl esters (CEs) in the chylomicron pool. The results indicate that there was a striking reduction in the CE fraction in the chylomicron pool of the patients with HLS than in the control subjects (**Figure 2**). This finding implies that the patients with HLS were forming fewer CE-rich chylomicron remnants, another indication that they had defective disposal of triacylglycerol from the chylomicron pool.

DISCUSSION

The results of this study reveal marked abnormalities in the ability of patients with HLS to metabolize dietary triacylglycerols. First, fasting concentrations of chylomicron- and VLDL-triacylglycerols were significantly higher in the patients with HLS, indicating that a substantial fraction of the triacylglycerols derived from the previous evening's meal had not been cleared even after a 10-h fast. Because our protocol was designed to
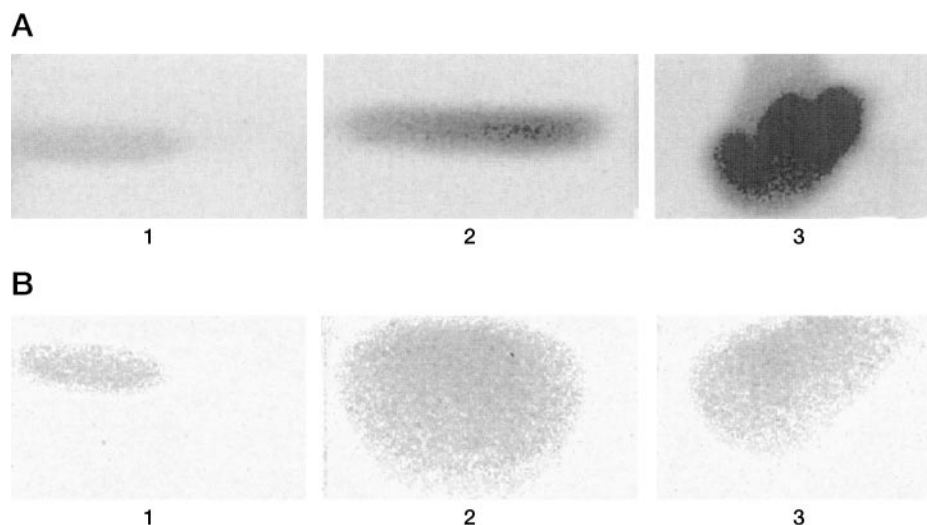

FIGURE 2. (A) Thin-layer chromatograph of the cholesteryl ester fractions in the plasma 8 h after consumption of a $[^{13}C_3]$tripalmitin meal. 1 = standard; 2 = HIV subject; 3 = control subject. (B) Thin-layer chromatograph of the triacylglycerol fractions in the plasma 8 h after consumption of a $[^{13}C_3]$tripalmitin meal. 1 = standard; 2 = HIV subject; 3 = control subject.

follow the kinetics of labeled dietary triacylglycerols for 8 h after a meal, we were also able to quantify marked defects in the disposal of dietary triacylglycerols and diet-derived FFAs and to infer the mechanisms underlying these defects. The patients with HLS had markedly lowered rates of disposal of chylomicron-triacylglycerol–derived $[^{13}C_1]$palmitate. In fact, patients with HLS retained 55% of the ingested fatty acid tracer in the chylomicron pool at the end of 8 h, whereas the control patients retained only 6% in this pool. Furthermore, of the 45% of the ingested tracer that was removed from the chylomicron fraction, only 18% was effectively stored in the patients with HLS, compared with 79% in the control subjects. The profound differences suggest that patients with HLS have severe defects in both the hydrolytic and trapping functions of adipocyte lipoprotein lipase (LPL). These defects in the postprandial metabolism of dietary lipids comprise an additional, independent mechanism of lipid dysregulation that contributes substantially to the persistent hypertriacylglycerolemia characteristic of HLS.

Both of the main findings of the present study, defective disposal of orally ingested $[^{13}C_3]$tripalmitin from the chylomicron pool and a 3-fold higher fasting plasma chylomicron-triacylglycerol concentration, suggest an impairment of the function of LPL. Several factors associated with HIV lipodystrophy could plausibly interfere with LPL expression or activity and thus lead to impaired fatty acid uptake by adipocytes. The mechanisms could include direct toxic effects of antiretroviral drugs on LPL activity (14–16), competitive inhibition of LPL by other lipoproteins, and inhibition of LPL by elevated FFA concentrations (17). Antiretroviral protease inhibitor drugs are known to impair LPL function. In vitro studies by Ranganathan et al (14) showed that saquinavir (and, to a lesser extent, indinavir) inhibits LPL activity significantly in 3T3-F442A and 3T3-L1 preadipocytes. Indinavir can also inhibit the expression of LPL and other SREPB-1c-dependent genes (15, 16). Competition for LPL between triacylglycerol-rich lipoproteins in the postprandial state could also contribute to defective LPL activity in patients with HIV lipodystrophy. LPL activity is required for the removal of triacylglycerols from both VLDL and chylomicron particles, hence elevated concentrations of VLDL-triacylglycerols could impair clearance of chylomicron-triacylglycerols through competitive inhibition (18–20). Finally, the elevated plasma concentrations of FFAs observed in HIV lipodystrophy (8) could inhibit LPL activity through product inhibition (17).

On the basis of our previous findings that patients with HLS have a primary defect in adipocyte function in the fasted state, resulting in accelerated lipolysis, excess FFA flux, and, hence, increased production of triacylglycerol-rich VLDL in the liver, we suggested that the underlying mechanism is defective regulation of adipocyte hormone-sensitive lipase (8). The excess release of triacylglycerol-rich VLDL into the circulation explains in part why patients with HLS are hypertriacylglycerolemic. The present data show that markedly defective disposal of chylomicron-triacylglycerols in the postprandial state adds to the severity and persistence of the hypertriacylglycerolemia and that the potential mechanism underlying these abnormalities could be defective LPL activity. Thus, the key to hypertriacylglycerolemia in HLS is a dysregulated fat cell with functional defects in both hormone-sensitive lipase and LPL, resulting in hyperlipolysis and impaired triacylglycerol clearance.

The possibility that a defect in LPL function underlies the abnormal chylomicron-triacylglycerol clearance in patients with HLS is supported by the results of the chylomicron subfraction analysis. For 8 h after ingestion of the test meal, the plasma chylomicron fraction in the patients with HLS was rich in triacylglycerols and relatively poor in CEs (ie, the chylomicron lipid composition had high triacylglycerol:CE), whereas the chylomicron fraction in the control subjects had a lower triacylglycerol content and a higher CE content (low triacylglycerol:CE). The difference suggests that the patients with HLS formed fewer chylomicron remnants than did the control subjects. This interpretation is supported by the finding of low plasma CEs in subjects with HLS, as measured by TLC (Figure 2A). These kinetic alterations in the disposal of triacylglycerols from the chylomicron-lipoprotein fraction are likely due to LPL dysfunction, because LPL activity preferentially removes triacylglycerols from triacylglycerol-rich chylomicrons that enter the plasma pool during a meal, resulting in triacylglycerol-depleted, relatively CE-rich chylomicron remnants. Consistent with this, Baril et al (21) have shown that patients with HLS have lowered activity of heparin-releasable plasma LPL.

Despite the markedly lowered disposal of chylomicron-$[^{13}C_3]$tripalmitin, the patients with HLS had more plasma non-esterified $[^{13}C_1]$palmitate than did the control subjects during the 8-h postmeal period. This apparent paradox can be explained by a review of the kinetics of LPL action with regard to the release and clearance of FFAs from chylomicron-triacylglycerols. Frayn et al (22) have reported that ≈50% of the FFAs released by LPL-mediated hydrolysis of triacylglycerols diffuse back into the plasma rather than enter into the adipoctye. Therefore, in normal health the "entrapment" function of LPL results in storage of only ≈50% of the FFAs released by the enzyme (22). Ware et al (23) have shown an impairment of the entrapment function of LPL in patients with HLS, whereby an even greater proportion of the released fatty acids enters into the plasma. They proposed that this defective entrapment of FFAs is responsible for the high fasting and postprandial plasma FFA concentrations seen in patients with HLS (23). The data in the present study indicate that, even though chylomicron-triacylglycerols were being hydrolyzed at a slower rate by the patients with HLS, a higher proportion of the $[^{13}C_1]$palmitate released from chylomicron-triacylglycerols was being left in the plasma instead of being taken up by the adipocytes. This explains the higher plasma concentration of nonesterified $[^{13}C_1]$palmitate 8 h after consumption of the $[^{13}C_3]$tripalmitin meal in the HLS subjects compared with the control subjects (Table 3). This would also explain why patients with HLS stored a smaller proportion of the dietary $[^{13}C_3]$tripalmitin than did the control subjects.

The higher plasma concentration of $[^{13}C_1]$palmitate 8 h after consumption of the $[^{13}C_3]$tripalmitin meal would also explain why the patients with HLS "paradoxically" oxidize more $[^{13}C_1]$palmitate than the control subjects, despite slower release from chylomicron-triacylglycerols. A higher content of plasma $[^{13}C_1]$palmitate means that the plasma nonesterified palmitate being taken up by organs and tissues for oxidation is more richly labeled with the $[^{13}C_1]$palmitate tracer. Consequently, the patients with HLS would have oxidized more $[^{13}C_1]$palmitate than control subjects 8 h after consuming the meal, even though the absolute rate of whole-body lipid oxidation may not be increased.

Abnormal chylomicron-triacylglycerol disposal in patients with HLS results in persistent postprandial lipemia. Postprandial lipemia is a strong predictor of coronary artery disease, even stronger than low HDL concentrations (24–27), hence the abnormal postprandial

lipid kinetics of patients with HLS add significantly to their cardiovascular risk. These data also have implications for treating the severe dyslipidemia of HLS. Traditionally, diets low in carbohydrate content (and, therefore, with a higher fraction of calories from fat) have been recommended for treating hypertriacylglycerolemia. However, HLS may be a condition in which a low-fat diet may have salutary effects. The recent recommendations of the National Cholesterol Education Program Adult Treatment Panel III are consistent with this notion and may be of benefit in treating the dyslipidemia of HLS (28, 29).

In conclusion, multiple pathogenic mechanisms acting in concert are responsible for marked and sustained hypertriacylglycerolemia in HLS. We have previously shown that the principal mechanisms in the fasted state are accelerated lipolysis with inadequate oxidative disposal of the released FFAs and, hence, increased VLDL-triacylglycerol production by the liver (8). In the postprandial state, a severe impairment of chylomicron-triacylglycerol disposal is likely due to a defect in LPL activity and entrapment of fatty acids by the adipocyte. Low-fat diets may be efficacious in treating postprandial lipemia and hypertriacylglycerolemia in patients with HLS.

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